

PCT

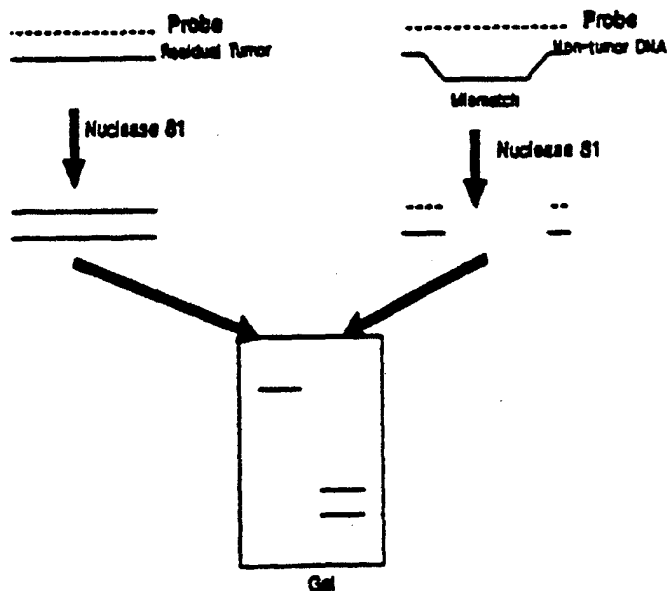
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US91/01547 (22) International Filing Date: 7 March 1991 (07.03.91) (30) Priority data: 501,496 30 March 1990 (30.03.90) US (60) Parent Application or Grant (63) Related by Continuation US 501,496 (CON) Filed on 30 March 1990 (30.03.90) (71) Applicant (for all designated States except US): CITY OF HOPE [US/US]; 1500 East Duarte Road, Duarte, CA 91010-0269 (US).	(72) Inventor; and (75) Inventor/Applicant (for US only) : BEN-EZRA, Jonathan [US/US]; 9161 Airdrome Street, Los Angeles, CA 90035 (US). (74) Agent: IRONS, Edward, S.; 919-18th Street, N.W., Suite 800, Washington, DC 20006 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>	

(54) Title: DETECTION OF MINIMAL RESIDUAL DISEASE IN LYMPHOID MALIGNANCIES



(57) Abstract

A method for detection of minimal residual disease in lymphoid malignancies by the following steps: (i) amplifying DNA from an original tumor using universal J and V primers in a polymerase chain reaction; (ii) preparing a single stranded probe from a discrete fragment of the amplification product of step (i); (iii) amplifying DNA from a test specimen suspected of containing residual disease with the same J and V primers in a polymerase chain reaction; (iv) hybridizing the products of steps (ii) and (iii); (v) subjecting the hybridization product to S₁ nuclease digestion; and (vi) determining the presence or absence in the digestion product of a sequence of substantially the same length as the probe prepared in step (ii).

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DETECTION OF MINIMAL RESIDUAL
DISEASE IN LYMPHOID MALIGNANCIES

This application is a continuation of United States application Serial No. 501,496 filed 30 March 1990.

FIELD OF INVENTION

This invention relates to a method for detecting minimum residual diseases (MRD) in lymphoid malignancies.

BACKGROUND OF INVENTION

One of the major problems in treating lymphoproliferative disease is the difficulty in detecting minimal residual disease (MRD). The sensitivity of the human eye in examining a bone marrow aspirate smear is not great. To detect a malignant population immunologically, approximately 10% of the cells have to belong to the malignant clone. Enhancement of the immunologic sensitivity, such as by K-S (Kolmogorov-Smirnov) curves, is sometimes possible, but even with this technique, the sensitivity can be as low as 5%, and this method will only work with surface immunoglobulin positive tumors. Molecular biology techniques, such as gene rearrangement studies by Southern hybridization, also have only enough sensitivity to detect a clone that comprises between 2-5% of a sample.

The polymerase chain reaction (PCR) has been used to detect MRD in lymphoproliferative lesions. (Stetler-Stevenson, et al., Blood 72:1822-1825 (1988); Crescenzi, et al. Proc. Nat. Acad. Sci. USA 85:4869-4873 (1988).) However, these studies have been most successful only in those lesions, such as follicular lymphoma or Burkitt's lymphoma, where a well-defined chromosomal translocation exists, and it is possible to amplify across this translocation. In these cases, if the translocation is present,

amplified material will be present; if the sought after translocation does not exist, no amplified genetic material will be generated.

Unfortunately, most lymphoid neoplasms do not have identifiable and will characterized chromosomal translocations. Therefore, attempts have been made to perform "generic" PCR, using universal primers. These studies utilize the fact that immunoglobulin and/or T-cell receptor genes undergo clonal somatic rearrangement in lymphoid neoplasms; if the DNA has not undergone rearrangement, the primers will be too far apart in the genome to form an amplifiable product by PCR. Several groups have attempted to detect MRD amplifying for either the T-cell receptor γ -chain gene, or for the immunoglobulin heavy chain gene. These latter methods require sequencing and then synthesis of a clonospecific oligonucleotide probe, steps which are not performed in every laboratory and can take weeks to perform.

The method of Yamada, et al. Proc. Nat. Acad. Sci. USA 86:5123-5127 (1989) involves amplifying a rearranged immunoglobulin heavy chain gene, which is present in all B- and pre-B-cell neoplasms, diseases which comprise more than 90% of lymphoid neoplasms. In this method, a universal J_H primer, one that will hybridize to all six J_H sequences, and a universal V_H primer, which recognizes the conserved framework region 3 (FR3) of the variable region, are used to amplify the rearranged immunoglobulin heavy gene, in which the J_H and FR3 regions are brought within 200 base pairs of each other. The amplified product is then sequenced, and a clonospecific oligonucleotide probe is synthesized that recognizes the unique CDR III region of the gene which is the portion of the

rearranged DNA that represents the highly variable junction the of the DNA splicing. Different tumors, because of the different nucleotide insertions in the junctional region, produced different length products.

The invention provides a simple method for the detection of MRD. The method is specific for a particular tumor without the need for sequencing the junctional region and subsequent manufacture of clonospecific oligonucleotide probes.

SUMMARY OF THE INVENTION

Pursuant to this invention both the original tumor specimen and the test specimen are amplified with identical PCR primers. The amplified product from the original tumor is used as a probe for the amplification product of the test specimen. Hybrids will form because both of the amplification products are produced with identical primers thus providing complementary end portions. However, internal sequences will hybridize only if the amplified test specimen includes MRD sequences since only such sequences will share the unique V-J-D rearrangement with the accompanying N-Segments that characterize the tumor of interest.

If this hybridization mixture is then subjected to S_1 nuclease digestion, only perfectly matched hybrids will remain intact, whereas those with mismatches will be cleaved into smaller pieces. When the digestion product is run on a gel and subjected to autoradiography, a band will appear at the position of the full length product only if residual tumor is present. This procedure is illustrated by Figure 1.

All of the methods used in the practice of this invention are well established techniques in molecular biology, and can be found in any molecular

biology laboratory manual. See e.g., Sambrook, J., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press (1989).

EXEMPLIFICATION OF THE INVENTION

The invention will be exemplified in part by reference to Figure 1. In general, the invention may comprise the following five steps:

1. Isolate DNA from the original tumor sample and amplify by PCR using universal J and V primers. See, Yamada, et al., supra.
2. Run the amplification product on a gel, cut a discrete band, and extract the DNA.
3. Prepare by any known means a labelled single stranded probe from the DNA extracted in step 2. For example, a single stranded labelled probe can be made directly using the transcription initiators of the plasmid and ³²S labelled nucleotides. The labelled product should be phenol-extracted, ethanol precipitated and resuspended.
4. Process specimen to be tested for MRD by repeating the procedure of step 1. The PCR product should be phenol-extracted, ethanol precipitated, and resuspended.
5. As generally illustrated by Figure 1, denature and hybridize the products of steps 3 and 4, preferably at about 60°C. Perform S₁ nuclease digestion, precipitate product and run on a polyacrylamide gel. If a tumor is present in the specimen a band as indicated in the left column of the Figure 1 gel will appear at the length of the discrete band cut in step 2, usually about 120-200 bp, depending on the specific tumor. If no tumor is present no such band will appear. Shorter bands produced by S₁ nuclease digestion elimination of a

mismatch region may appear as illustrated b the right column of the Figure 1 gel.

CLAIMS:

1. A method for detection of residual disease in lymphoid malignancies which comprises

(i) amplifying DNA from an original tumor using universal J and V primers in a polymerase chain reaction;

(ii) preparing a single stranded probe from a discrete fragment of the amplification product of step (i);

(iii) amplifying DNA from a test specimen suspected of containing residual disease with the same J and V primers in a polymerase chain reaction;

(iv) hybridizing the products of steps (ii) and (iii);

(v) subjecting the hybridization product to S_1 nuclease digestion; and

(vi) determining the presence or absence in the digestion product of a sequence of substantially the same length as the probe prepared in step (ii).

2. A method as defined by claim 1 in which the digestion product is subjected autoradiograph to determine the presence or absence in the digestion product of said sequence of substantially the same length as probe prepared in step (ii).

3. A method as defined by claim 1 in which said probe prepared in step (ii) is prepared from a DNA fragment about 120 to about 200 base pairs in length.

4. A method as defined by claim 1 in which the amplification in steps (i) and (ii) is accomplished by the polymerase chain reaction.

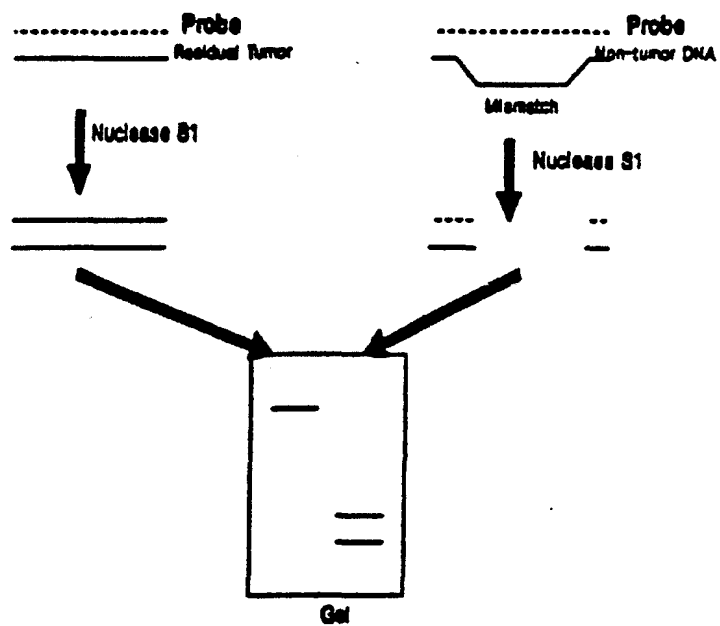


FIGURE 1

INT. NATIONAL SEARCH REPORT

International Application No. **PCT/US 91/01547**

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) According to International Patent Classification (IPC) or to both National Classification and IPC <div style="text-align: center; margin-top: 5px;"> IPC (5): C12 Q 1/68 U.S. cl.: 435/6 </div>						
II. FIELDS SEARCHED <div style="text-align: center; margin-top: 5px;"> Minimum Documentation Searched¹ </div> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 5px;"> <tr> <td style="width: 25%; padding: 5px;">Classification System</td> <td style="width: 75%; padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px; text-align: center;">U.S. CL</td> <td style="padding: 5px; text-align: center;">435/6</td> </tr> </table> <div style="text-align: center; margin-top: 5px;"> Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched⁴ </div>			Classification System	Classification Symbols	U.S. CL	435/6
Classification System	Classification Symbols					
U.S. CL	435/6					
CA. Biosis. Medline. Biotech Abs. Current Biotech.						
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁵						
Category ⁶	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³				
X	Proceedings of the National Academy of Science, vol. 86, Issued July 1989, Yamada et al, "Detection of minimal disease in hematopoietic malignancies of the B-cell lineage by using third-complementarily-determining region (C2R-III)-specific probes" pp5123-5127, see all document.	1-4				
Y	Proceedings of the National Academy of Science, vol. 85, issued July 1988, Crescenzi et al, "Thermoestable DNA polymerase chain amplification of t(14;18) Chromosome breakpoints and detection at minimal residual disease" pp 4869-4873, see all document.	1-4				
Y	Blood, vol. 72, No. 5, issued November 1988, "Detection of Oeult Follicular Lymphoma by specific DNA amplification" pp 1822-1825, see all document.	1-4				
Y	Cell, vol. 27, issued December 1981, Rauetch et al "Structure of the human immunoglobulin u-locus: characterization of	1-4				
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁴ Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>						
IV. CERTIFICATION						
Date of the Actual Completion of the International Search : <div style="text-align: center; margin-top: 10px;"> 10 April 1991 </div>		Date of Mailing of this International Search Report : <div style="text-align: center; margin-top: 10px;"> 21 JUN 1991 </div>				
International Searching Authority : <div style="text-align: center; margin-top: 10px;"> ISA/US </div>		Signature of Authorized Officer : <div style="text-align: center; margin-top: 10px;"> Miguel Escallon </div>				

FURTHER INFORMATION CONTAINED FROM THE SECOND SHEET

	embryonic and rearranged J and D genes." pp 583-591, see pp 586, col. 2 and pp. 588, col. 2	
Y	EMBO Journal, vol 7, No. 3 issued 1988, Berman et al, "Content and organization of the human Ig vh Locus: definition of three new Vh families and linkage of the Ig Ch Locus", pp 727-738, see all document.	1-4
Y	US, A, 4,683,195 (Mullis et al.) 28 July 1987, see all document.	1-4

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹, specifically:

3. ☐ Claim numbers because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.